Pak. J. Bot., 42(6): 4329-4333, 2010.

EFFECT OF OILSEED CAKES ALONE OR IN COMBINATION WITH *TRICHODERMA* SPECIES FOR THE CONTROL OF CHARCOAL ROT OF SUNFLOWER (*HELIANTHUS ANNUS* L.)

MUHAMMAD ANIS, M. JAVED ZAKI AND SHAHNAZ DAWAR

Department of Botany,
University of Karachi, Karachi-75270, Pakistan

Abstract

Seed treatment with oil seed cakes like Cotton cake, Mustard cake (Black and yellow) and Taramera cake alone or in combination with *Trichoderma harzianum* and *T. resei* significantly reduced colonization of roots by charcoal rot fungus (*Macrophomina phaseolina*) and significantly increased growth of sunflower (*Helianthus annus* L.) plants. Highest reduction in charcoal rot of sunflower was observed when seeds of sunflower were coated with cotton cake and *T. resei* followed by Taramera cake and *T. harzianum* and Mustard cake in combination with *T. harzianum.*

Introduction

Seed coating is applied as finely ground solids or liquids containing dissolved or suspended solids to form a layer covering the seed coat (Scott, 1989). This helps materials to be applied in such a way that they affect the seed or soil at the seed-soil interface and is the most reliable way to apply the biocontrol agent in close proximity with the germinating seed (Powell, 1992; Paulitz, 1992). The process of seed coating usually involves the use of adhesives, also known as stickers, to bind material to the surface of the seeds (Scott, 1989; Taylor & Harman, 1990). Dawar *et al.*, (2008) used different coating agents like mollases, glucose, gum arabic and sugar solution but gum arabic reported to be the best of all in controlling *M. phaseolina, R. solani* and *Fusarium* species on okra and sunflower.

The causal agent of charcoal rot is *Macrophomina phaseolina* (Tassi) Goid, a soil, seed and stubble borne fungus which infect the root and lower stem of over 500 cultivated and wild plant species (Indera *et al.*, 1986). Its infection on sunflower was first reported from Sri Lanka in 1927. Afterwards it was reported from many parts of the world including Pakistan (Dhingra & Sinclair, 1978; Mirza & Beg, 1983).

Organic amendments are generally used for improving crops, increasing agricultural productivity and suppressing soil borne diseases (Stone *et al.*, 2003). Beside a wide variety of organic matters that have been tested as organic amendments for managing plant pathogens are oil seed cakes which decreased the population of soilborne pathogens (Sharma *et al.*, 1995). Oil seed cakes are by-products obtained after oil extraction from the seeds. Oil cakes are of two types, edible and non-edible. Non- edible oil cakes such as castor cake, neem cake are used as organic nitrogenous fertilizers, due to their NPK content. Some of these oil cakes are found to increase the nitrogen uptake of the plant and protect the plants from soil nematodes, insects, and parasites (Ramachandran *et al.*, 2007). It is observed that several antimicrobial by-products (e.g. organic acids, hydrogen sulfide, phenols, tannins and nitrogenous compounds) are released during the decomposition of organic amendments, or synthesized by microorganisms involved in such degradation (Rodriguez-Kabana *et al.*, 1995). Different organic substrates like Datura powder (Shahwar *et al.*, 1994), *Avicennia marina* (Tariq *et al.*, 2006), *Eucalyptus* sp., (Dawar *et al.*, 2007) have shown promising results in controlling root infecting fungi like *M. phaseolina, R.*

solani and *Fusarium* spp. Dawar *et al.*, (2007) reported that sea weeds (*Melanothanus afaqhusainii, Padina tetrastromatica, Cytoclonium purpuraeum* and *Hypnea valentiae*) used alone or in combination with Bacteria (*Rhizobium meliloti, Pseudomonas aeruginosa, Bacillus subtilis*) significantly suppressed root infecting fungi on mash bean and sunflower. Similarly Malik *et al.*, (2005) assessed *T. harzianum* alone or in combine application with different organic substrates like rice grain, sorghum grain, millet grain and saw dust at different days for reducing the infection of root infecting fungi.

In the present study, sunflower seeds were coated with oil seed cakes of Mustard (yellow and black), Taramera, Cotton cake alone or in combination with *T. harzianum* and *T. resei*. The aim of the study was to observe the effect of biocontrol agents and oil seed cakes alone or in combination on growth parameters and colonization of *M. phaseolina* on sunflower.

Materials and Methods

Collection of materials: Two *Trichoderma* species like *T. harzianum* (KUMCC-65) and *T. resei* (KUMCC-28) were obtained from Karachi University Mycological Culture Collection (KUMCC) and were maintained on Potato Dextrose Agar (PDA). Oil seed cakes like Cotton, Taramera and Mustard cakes (Black & yellow) were purchased from local market and ground in electric grinder.

Effect of oil seeds cakes: Sunflower seeds (*Helianthus annus* L.) var. Hysun 33 were surface sterilized with 1 % $Ca(OCl)_2$ for three min., rinsed thoroughly in running tap water and dried aseptically. The seeds were treated with mycelial and sclerotial suspension of *M. phaseolina*, and oil seed cakes of mustard (black & yellow), cotton, taramera separately using 2 % gum arabic solution as a sticking material. Seeds after coating were air dried under laminar flow hood.

Effect of oil seed cakes with *Trichoderma* species: 300 g soil which were obtained from experimental plots of Department of Botany, University of Karachi were placed in plastic pots of 8 cm diameter. The soil contained natural infestation of 2-4 sclerotia of *M. phaseolina* per g of soil (Sheikh & Ghaffar, 1975). The pH of soil calculated to be 8.2, moisture holding capacity 24.04% (Keen & Raczkowski, 1922), total nitrogen 1.5% (Mackenzie & Wallace, 1954) and total organic matter 23%. Five seeds of sunflower after treatment with oil seed cakes of mustard (black and yellow), cotton and taramera with or without *Trichoderma* species (*T. harzianum, T. resei*) were sown in each pot separately. The pots without treated seeds served as control and there were three replicates of each treatment. Pots were kept in a randomized fashion at the screen house of Department of Botany, University of Karachi. After 30 days of seedling emergence, the plant growth parameters in terms of shoot length, shoot weight, root length, root weight was recorded. For the colonization %, one cm long root pieces after washing in running tap water were plated on potato dextrose agar (PDA) containing streptomycin (0.2 g/l) and penicillin (100,000 units/l).

Analysis of data: Data were analysed using one way analysis of variance (ANOVA). The treatment means were compared as Least significance difference ($LSD_{0.05}$) (Sokal & Rohlf, 1995).

Results

Effect of oil seed cakes against *M. phaseolina* on sunflower: *M. phaseolina* when used alone as seed coating to sunflower were found to increase the colonization % as compared to control (without *M. phaseolina)* but oil seed cake, mustard cake (black) showed effective result on plant growth and incidence of root rot. Plant length and weight were significantly ($p<0.05$) increased (30 cm), (4 g) in contrast to control (23.25 cm), (2.7 g). Colonization % of charcoal rot fungus also significantly ($p<0.05$) decreased when mustard cake (black) were used (Table 1). Vigor index of plant also increased when mustard cake (black) was used as compared to *M. phaseolina* alone (Table 1).

Effect of oil seed cakes and *Trichoderma* species for the control of *M. phaseolina*: Two *Trichoderma* species and four different types of cakes used alone or in combination with each other were used in the present study to observe its effect on plant growth. Sunflower seed when treated with mustard cake (black) showed significant ($p<0.05$) increase in shoot length (19.5 cm) as compared to control (12.5 cm) (Table 1). Weight of root, shoot and root length showed significant ($p<0.05$) result over control when cotton cake was used in combination with *T. resei.* Mustard cake (black and yellow) when used alone as seed treatment showed increased growth parameters (Table 2). Use of cotton cake in combination with *T. resei* were found to be the best in reducing the colonization % of *M. phaseolina* on roots of sunflower (6%) whereas taramera cake in combination with *T. harzianum* (7%) were also significantly ($p<0.05$) helpful in controlling *M phaseolina* colonization of sunflower roots (Table 1). Observation of the present study showed that *T. harzianum* and *T. resei* when used alone as sunflower treated seed were less effective in controlling the *M .phaseolina* colonization.

Discussion

Seed treatment were found to be an attractive method for introducing biocontrol agents and organic matter to soil environment as it protects the seed from seedborne fungi and helps in germination, establishment of healthy seedling (Chang & Kommedahl, 1968). Present results indicate an increase in plant growth parameters when cotton cake is applied to seed of sunflower in combination with *T. resei* and mustard cake (black) applied to sunflower seed alone. A decrease in the colonization % of *M. phaseolina* was observed when cotton cake was used with *T. resei* and taramera cake with *T. harzianum.* Dawar *et al*., (2008) used different coating materials for coating *Bacillus thuringiensis, Rhizobium meliloti, Aspergillus niger* and *Trichoderma harzianum*, but effective result in improvement of plant growth was observed when *T. harzianum* was coated on okra and sunflower seeds. There are two main categories of seed treatment like protectant (contact on the seed surface) and systemic (within plants). Protectants found to control pathogens that reside on the seed surface while systemic seed treatments control seed borne fungi that reside within the seed or infect the seed surface (Taylor & Harman, 1990). Sharma *et al.*, (1995) suggested that there is a reduction in yield loss caused by dry root rot of guar (*Cyamopsis tetragonoloba* (L.) Taub.) and wilt of cumin (*Cuminum cyminum* L.) by applying mustard cake in fields infested with *M. phaseolina* and *F. oxysporum* f. sp. *cumini.*

It would suggest that applying cotton cake and mustard cake to seed in combination with *Trichoderma* species were found to be beneficial in controlling *M. phaseolina* infection on sunflower.

Table 1. Effect of seed treatment with oil seed cakes for the control of *Macrophomina phaseolina* and growth of sunflower (*Helianthus annus* L.).

Treatments	Germination %	Plant length (cm)	Plant weight (g)	Colonization %	Vigor Index
Control	60a	23.25 a	2.7c	03d	1395 b
Macrophomina phaseolina (MP)	40c	16.75 b	2.3c	80a	670 f
MP+ Mustard cake (black)	60a	30 ab	4.4a	20b	1800 a
MP+Mustard cake (yellow)	50b	23.75 ab	3.6ab	40c	1187.50 d
MP+Cotton cake	50b	25.75 ab	4.1ab	20b	1287.50 c
MP+Taramera cake	45bc	22.75ab	3.5b	40c	1023.25 e
$LSD_{0.05}$	**8.5933**	**9.2700**	**0.7889**	**6.9661**	**38.8500**

Same letters in each column are not significantly different according to Duncan's Multiple Range Test.

Table 2. Effect of seed treatment with oil seed cakes alone or in combination with *Trichoderma* species for the control of *Macrophomina phaseolina* and growth of sunflower (*Helianthus annus* L.).

Treatments	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Colonization %
Control	12.5 cde	5 b	1.4 cd	0.8 de	93 a
Trichoderma harzianum	15.12 abcd	5.75 ab	1.5 bcd	0.96 cde	53 b
T. resei	14.77 abcd	6 ab	1.5 bcd	1.13 bcd	52 b
Mustard cake (black)	19.5 a	6.25 ab	1.7 abcd	1.5 bc	30 c
Mustard cake (yellow)	16.25 abcd	6.5 ab	1.7 abcd	1.45 bcd	28 c
Cotton cake	17.75 ab	6 ab	1.5 bcd	1.2 bcd	26 cd
Taramera cake	16 abcd	6 ab	1.3 d	1.13 bcd	20 cde
T. harzianum+ Mustard cake (black)	17.5 abc	7 ab	1.4 cd	1 cde	10 ef
T. harzianum+Mustard cake (yellow)	14 bcde	6 ab	1.5 bcd	1.2 bcd	30 c
T. harzianum+ Cotton cake	16 abcd	7.25 ab	1.7 abcd	1.2 bcd	13 def
T. harzianum+Taramera cake	14 bcde	8.25 ab	2.3 ab	1.7 ab	07 ef
T. resei+ Mustard cake (black)	14 bcde	6.75 ab	1.4 cd	1.5 bc	10 ef
T. resei+ Mustard cake (yellow)	12.25 de	6.5 ab	1.6 bcd	01 cde	26 cd
T. resei +Cotton cake	15 abcde	8.75 a	2.5 a	2.1 a	06 f
T. resei +Taramera cake	10.5 e	6.5 ab	1.2 d	0.45 e	20 cde
$LSD_{0.05}$	**4.3325**	**2.7975**	**0.7508**	**0.5472**	**12.0322**

Same letters in each column are not significantly different according to Duncan's Multiple Range Test.

References

Bhutta, A.R. 1997. *Biological studies on some fungi associated with sunflower in Pakistan.* Ph.D thesis, Plant Pathology Department, Sindh Agriculture University, Tandojam.

Chang, I. and T. Kommedahl. 1968. Biological control of seedling blight of corn by coating kernels with antagonistc microorganisms. *Phytopathology,* 58: 1395-1401.

Dawar, S. S. Hayat, M. Anis and M.J. Zaki. 2008. Effect of seed coating materials in the efficacy of microbial antagonists for the control of root rot fungi on okra and sunflower. *Pak. J. Bot.*, 40(3): 1269-1278.

Dawar, S., S. Arjumand., M. Tariq and M.J.Zaki. 2007. Use of sea weed and bacteria in the control of root rot of mash bean and sunflower. *Pak. J. Bot.,* 39 (4): 1359-1366.

Dawar, S., S. Younus., M. Tariq and M.J.Zaki. 2007. Use of *Eucalyptus* sp., in the control of root infecting fungi on mung bean and chick pea. *Pak. J. Bot.*, 39 (3): 975-979.

Dhingra, O.D. and J.B. Sinclair. 1978. *Biology and Pathology of Macrophomina phaseolina*. Univ. Federal deViscosa, Brazil, p.166.

Indera, K., T. Singh, C.C. Machado and J.B. Sinclair. 1986. Histopathology of soybean seed infection by *Macrophomina phaseolina*. *Phytopathology*, 76: 532-535.

Keen, B.A and H. Raczkowski. 1922. Clay contents and certain physical properties of soil. *J.Agric., Sci.,* 11:441-449.

Mackanzie, H.A. and H.S. Wallace. 1954. The Kjeldahl determination of nitrogen. A critical study of digestion conditions, temperature, catalyst and oxidizing agents. *Aust. J.Chem.,* 7:55-70.

Malik, G., S. Dawar, A. Sattar and A. Dawar, 2005. Efficacy of *Trichoderma harzianum* after multiplication on different substrates in the control of root rot fungi, *Int. J. Biol. Biotech*., 2(1): 237-242.

Mirza, M.S. and A. Beg. 1983. Diseases of sunflower in Pakistan in 1982. *Hellia,* 6: 55-56.

Paulitz, T.C., O. Anas and D.G. Fernando. 1992. Biological control of *Pythium* damping-off by seed treatment with *Pseudomonas putida*: relationship with ethanol production by pea and soybean seeds. *Biocontrol. Sci. Technol*., 2: 93-201.

Powell, K. 1992. Is biological control the answer to sustainable agriculture? *Chem. Ind.,* 5: 168-170.

Ramachandran, S., S.K. Singh, C. Larroche, C.R. Soccol and A. Pandey. 2007. Oil cakes and their biotechnological applications – A review. *Biores Techno.,* 98: 2000-2009.

Rodriguez-Kabana, R., V. Estaun, J. Pino-Chet and O. Marfa. 1995. Mixtures of olive pomace with different nitrogen sources for the control of *Meloidogyne* spp. on tomato. *Suppl. J. Nematol.* 27: 575-584.

Scott, J.M. 1989. Seed coatings and treatments and their effects on plant establishment. *Adv. Agron.* 42: 43-83.

Shahwar, D., M. Abid, M. Attaur Rehman, M. A. Maqbool and M.I. Choudhary. 1995. Nematicidal compounds from *Datura fastuosa*. In: *Proc. 19th IUPAC Symposium on the chemistry of Natural Products.* pp 171-179. (ed) Atta-ur-Rehman, M.A.Choudhary and M.S. Sheikhani. HEJ Res. Inst. of Chemistry, University of Karachi, Karachi 75270- Pakistan. pp 409.

Sharma, S.K., R.K. Aggarwal and S. Lodha. 1995. Population changes of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* in oil-cake and crop residue amended sandy soils. *Applied Soil Ecology*, 2(4): 281-284.

Sheikh, AH. And A.Ghaffar. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot*., 7: 13-17.

Sokal, R.R. and F.J. Rohlf. 1995. *Biometry: The Principles and Practices of Statistics in Biological Research.* Freeman, New York, pp. 887.

Stone, A.G., G.E. Vallad, L.R. Cooperband, D.R. Rotenburg, H.M. Darby, W.R. Stevenson and R.M. Goodman. 2003. Impact of annual organic amendment on disease incidence in a three year vegetable rotation. *Plant Disease,* 87: 1037-1042.

Tariq, M. S. Dawar, F.S. Mehdi and M.J. Zaki, 2006. Use of *Avicennia marina* in the control root infecting fungi in okra and mash bean. *Pak . J. Bot.,* 38 (3): 811-815.

Taylor, A.G. and G.E. Harman. 1990. Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathol.,* 28: 321-339.

(Received for publication 17 February 2010)